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(71) Applicants and
(72) Inventors: **TEERI, Teemu** [FI/FI]; Porttitie 17 B, FIN-02180 Espoo (FI). **HELARIUTTA, Yrjö** [FI/FI]; Oskelantie 8 A 5, FIN-00320 Helsinki (FI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KOTILAINEN, Mika** [FI/FI]; Hiihtomäentie 44 B 26, FIN-00800 Helsinki (FI). **MEHTO, Merja** [FI/FI]; Rukkilanrinne 4 C 20, FIN-00410 Helsinki (FI). **PÖLLÄNEN, Eija** [FI/FI]; Juustenintie 3 E 60, FIN-00410 Helsinki (FI). **ELOMAA, Paula** [FI/FI]; Lammaspolku 1 A 25, FIN-01710 Vantaa (FI).

(74) Agent: **BORENIUS & CO OY AB**; Kansakoulukuja 3, FIN-00100 Helsinki (FI).

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(54) Title: METHODS AND GENE PRODUCTS FOR ALTERING THE SHAPE OF CELLS AND ORGANS IN PLANTS

(57) Abstract: The present invention is related to GEG-like genes and their gene products capable of controlling the direction and dimensions of cell growth. These genes and gene products can be applied for altering the size shape of plant cells and/or organs. An expansion in either radial growth or longitudinal growth of the plant cell is desirable. Said genes and gene products are useful in agriculture, horticulture and forestry for manufacturing renewable plant-derived raw-material structures more convenient, advantageous and applicable in the respective application.

Methods and Gene Products for Altering the Shape of Cells and Organs in Plants

The Technical Field of the Invention

The present invention is related to a method for adjusting the shape of plant cells and plant organs. The invention is also related to GEG cDNA or GEG-like nucleic acid sequences and their products useful in horticulture, agriculture and forestry for manufacturing renewable plant-derived raw-materials with structures more convenient, advantageous and feasible for the respective application.

The Background of the Invention

The highly invariable shape and size of floral organs, suggests that their shape is under strict developmental control. During flower development, the final shape of the floral organs typically occurs after mitotic activity has basically ceased, indicating that cell expansion plays an important role in determining organ shape (Pyke, K. A., et al., J. Exp. Bot. 42, 1407-1416, 1991; Tsuge, T., et al., Development 122, 1589-1600, 1996). Mutations for leaf development (Tsuge, T,. et al., Development 122, 1589-1600, 1996) and several cell expansion mutations for root development in *Arabidopsis* (Aeschbacher, R. A., et al., Genes & Development 9, 330-340, 1991; Hauser, M.-T., et al., Development 121, 1237-1252, 1995) point to an interrelationship between the longitudinal and radial dimensions of the cell. Mutants have been shown to exhibit restricted expansion in leaf-width direction and enhanced expansion in leaf thickness. In some plants morphology is affected in one direction only and in others in several directions.

Because plants are one of the most important renewable resources in nature, it would be beneficial to be able to control the direction and dimension of cell growth and organ shape in plants. The capability of controlling and regulating the direction and dimension of cell growth could be utilized to provide the most convenient and advantageous shape or structure for any desired application in agriculture, horticulture and forestry. Either increased longitudinal, e.g. longer cells or fibers could be beneficial for some applications in pulp and paper industry, whereas radial cell growth could be desired in many other applications. So far the molecular and genetic regulation of cell expansion is not well understood and especially the molecular control of organ shape during flower development is still largely unknown.

The objectives of the present invention is to provide new methods for altering or adjusting the shape of plant cells and organs by controlling and/or regulating the direction, both in longitudinal and radial direction, as well as the magnitude of cell growth.

Another objective of the present invention is to provide means carrying out said method. The means are GEG cDNA or GEG-like nucleic acid sequences, including promoters of said GEG or GEG-like genes or nucleic acid sequences capable of directing foreign genes or heterologous nucleic acid sequences in desired direction. The GEG-like gene products of said nucleic acid sequences are useful in agriculture, horticulture and forestry for manufacturing renewable plant-derived raw-materials with structures more convenient, advantageous and feasible for the respective application.

Especially preferred objectives of the present invention in forestry is to enable modification of the length and/or breadth of the fibers obtainable for example from wood. In agriculture or horticulture one of the desired objectives is to provide shorter and broader stems which are more stable or resistant to crop flattening by rain, etc.

In horticulture the objective could be to modify the shape of flowers and/or leaves to provide more decorative forms or longer flower bearing stems as well as other desirable properties in the plants in question.

The Summary of the Invention

The present inventions provides a solution to said problems by providing new methods for controlling the direction of cell growth and organ shaping in plants. The method and the products and means utilized in said method are as defined in the claims of the present invention.

A Short Description of the Drawings

Figure 1. RNA gel blot hybridisation analysis showing the tissue specificity of *GEG* expression.

Figure 2. Analysis of *GEG* expression during corolla and carpel development.

Figure 3. Analysis of *GEG* expression in carpel and corolla by *in situ* hybridization.

Figure 4. Biometric analysis of corolla and carpel length and width development.

Figure 5. Cell length and width in distal and central regions of ray floret corolla just prior and after the opening (stages 7+ and 8, respectively).

Figure 6. Comparison of the ray floret corollas of a non-transformed line (wt) with that of an m_3 plant constitutively expressing *GEG* before (stage 7), during (7.5), and after (9) the opening of ray florets.

Figure 7. Analysis of corolla length and width for four lines constitutively expressing *GEG* and the control lines.

Figure 8. Analysis of the effects of constitutive *GEG* expression on corolla epidermal cells.

Figure 9. Cell lengths and widths in the same regions as described in Figure 8 of m_1 and m_3 lines constitutively expressing *GEG* and a control line (wt). In plants constitutively expressing *GEG*, cell length was reduced but no difference in cell width could be measured.

Figure 10. Scanning electron microscopy of the epidermis of stylar part of the carpel 300 m below the stigma. In an m_1 line constitutively expressing *GEG*, cell length was reduced and the width was increased compared to control line.

Figure 11. Analysis of the effects of constitutive *GEG* expression on epidermal cells of the carpel style.

Figure 12. Exogenous gibberellic acid application upregulates *GEG* expression in ray floret corollas

Figure 13. The promoter sequence, the cDNA sequence and the amino acid sequence of the GEG-gene product are shown as well as the points of initiation and termination of the sequences pertinent in the present invention.

The Detailed Description of the Invention

Definitions

In the present invention the terms used have the meaning they generally have in the fields of conventional plant breeding, plant biochemistry and production of transgenic plants, including recombinant DNA technology as well as agriculture, horticulture and forestry. Some terms, however, are used with a somewhat deviating or broader meaning in this context. Accordingly, in order to avoid uncertainty caused by terms with unclear meaning some of the terms used in this specification and in the claims are defined in more detail below.

The term "GEG" for "gerbera *GAST1*-like gene" means an isolated and essentially purified cDNA sequence (*Gerbera hybrida* homolog for the gibberellic acid stimulated transcript 1 [*GAST1*], from tomato) obtainable from a library representing late stages of corolla development in *Gerbera hybrida*.

The term "GEG-like nucleic acid sequences" means nucleic acid sequences homologous to said *GEG*-gene or cDNA. The "GEG-like nucleic acid sequences" are characterized by a nucleic acid sequence encoding "GEG-like gene products" having an amino acid sequence substantially homologous with the C-terminal domain (SEQ ID NO:1:) of the gene product of GEG (SEQ ID NO:2:). Said "GEG-like nucleic acid sequences" are further substantially similar at nucleotide level with the GEG cDNA (SEQ ID NO:3:). In its broadest aspect GEG-like nucleic acid sequences include the GEG-promoter SEQ ID NO:4: as well as substantially homologous promoters, which are capable of directing a foreign gene or a heterologous nucleic acid sequence in the same way as GEG is directed by its native promoter in corolla and styles.

Said "GEG-like nucleic acid sequences" are further characterized by the capacity of spatiotemporal controlling of the plant cell growth by alternatively increasing and/or decreasing the cell growth in various directions or by directing said function.

Typical examples of such GEG-like nucleic acid sequences include isolated, essentially purified nucleotide sequences obtainable, for example, by differential hybridization from a group of plant genes having a high similarity with the GEG cDNA from *Gerbera hybrida*.

The term "DNA construct for altering the size and shape of plant cells or plant organs" means any suitable vectors and/or DNA constructs comprising at least one GEG-like nucleic acid sequences combined with optional promoters, enhancers, signal sequences for inserting, targetting, controlling the size and shape of plants cells and plant organs.

The term "GEG gene product" means an amino acid sequence with the deduced amino acid sequence (SEQ ID NO:2:) sharing a high similarity with previously characterized putative cell wall proteins encoded by GEG-like nucleic acid sequences. The "GEG-like gene products" are polypeptides characterized by having an amino acid sequence comprising amino acid sequences substantially homologous with SEQ ID NO:2:, which are further characterized by having a highly conserved C-terminal domain, with one or more invariable cysteine residues. The "GEG-like gene products" are further characterized by the capacity of spatiotemporal control of cell growth, which can be determined by methods disclosed in the examples.

The term "GEG-like gene products" include in addition to said GEG-gene product, products obtainable by constitutive or induced expression of gibberellic acid-inducible genes, namely GAST1 of tomato, GIP (for gibberellin-induced gene) of petunia and the GASA (for GA-stimulated in *Arabidopsis*) gene family of *Arabidopsis*. *GEG* and GEG-like genes, the expression of which can also be induced by application of exogenous gibberellic acid (GA_3) plays a role in phytohormone-mediated cell expansion. The use of a GEG-like gene for manufacturing plants having the capability of controlling the direction and dimensions of cell growth and altering the shape of plant cells and/or plant organs.

The term "spatiotemporal control" means that said GEG-like nucleic acid and their expression products are capable of controlling, i.e. regulating and/or adjusting the cell growth by alternatively, increasing and/or decreasing or inhibiting the cell growth in various directions, including longitudinal and/or radial direction with adjustable, advantageous time intervals.

The term "substantially homologous" means that the GEG-gene product have a homology of at least 40 %, preferably at least 50 %, most preferably at least 55 % at amino acid level.

The term "defined hybridization conditions" means any hybridization conditions varying between the conditions of 58 °C, 2xSSC and 58 °C, 0.2XSSC. The upper limit allows

the capture of genes closely related with Gerbera and the lower limit of at least 58 °C, 0.2XSSC allows the capture of the GEG-gene as such, especially when the part of the nucleic acid sequence encoding the homologous C-terminal domain is excluded.

The term "plant cell line" means a cell line into which a GEG-like gene is inserted by *per se* known methods or is a cell line capable of expressing a GEG-like gene product having the capacity of controlling the direction and dimensions of cell growth, especially increasing the radial cell growth and inhibiting the longitudinal cell growth.

The term "transgenic plant" means a plant into the cells of which at least one GEG-like gene has been introduced or integrated and which cells are capable of expressing a GEG-like gene product having the capacity of controlling the direction and dimensions of cell growth, especially increasing the radial cell growth and inhibiting the longitudinal cell growth or vice versa.

The terms "GEG-like gene products and derivatives thereof" cover all possible splice variants of the GEG-product, including truncated, complexed as well as derivatized forms of said GEG-product, which still have the capacity of spatiotemporal control of cell growth in plants.

As a conclusion "GEG-like gene product" in its broadest aspect in the present invention, covers not only normal GEG-like molecules including their isoforms of different origin, as separate entities or in any combinations. The term covers all listed gene products in their active forms and in any combinations of said forms as well as fragmented, truncated, derivatized and/or complexed forms thereof, which fulfill the prerequisites defined in the previous paragraph.

The term "isoform" refers to the different forms of the same protein, which originate from different sources, e.g. different species of plants. In the present invention the term includes fragments, complexes and their derivatives. For example, GEG-like gene products are generated e.g. by the cleavage. Different reactions, including different enzymatic and non-enzymatic reactions, proteolytic and non-proteolytic, are capable of creating a truncated, derivatized, complexed form of the said GEG-gene product. They are incorporated in the present invention as long as they fulfill the prerequisite of capacity for spatiotemporal control of plant cell growth.

The term "altering" means capacity of spatiotemporal control of plant cell growth, i.e.

adjusting by molecular regulation the direction and dimensions of cell growth and the organ shape in plants, especially during flower development.

The term "manufacturing plants with spatiotemporal control of cell growth" means that the plants are transgenic plants produced by incorporating (inserting) a DNA-construct or vector carrying one or more nucleic acid sequences of the present invention into a plant cell, which can be induced to express said insert by administration of gibberellic acid (GA_3) or auxin or is constitutively expressing said insert. Said plant cells are capable of spatiotemporally directing cell growth in variable directions in order to allow reshaping of organs in plants.

The GEG-like gene products expressed by the GEG-like nucleic acid sequences of the present invention are, accordingly, useful for altering or adjusting the size and shape of plant cells or plant organs to its respective application.

The GEG-like nucleic acid sequence as well as their expression products can be used for manufacturing plant cells or plants with modifiable size and/or shape by directing the plant cell growth, in various, alternatively, in longitudinal and/or radial direction to obtain the size and shape of plant cells or plant organs, which is best suited to its respective application in agriculture, horticulture and/or forestry.

It is especially desirable if the longitudinal growth of plant cells is increased and allows the production of longer fibers for applications in pulp and paper industry, or production of longer floral stems in horticulture, or if the longitudinal growth of plant cells is decreased and allows production of crop plants with shorter stem and increased resistance and which prevents flattening of the crop in agricultural and horticultural applications.

The General Description of the Invention

Recently, knowledge about the control of cell growth in plants has accumulated with increasing rapidity, but the molecular control of organ shape during flower development is still largely unknown. The *GEG*-like gene/protein family shares several features that may suggest a role for GEG in regulating cell expansion. The "GEG-like genes and their gene products", both novel and previously known, are useful for manufacturing plants having the capability of controlling the direction and dimensions of cell growth and altering the shape of plant cells and/or plant organs.

By using differential hybridization techniques, the present inventors isolated a cDNA designated *GEG* (*Gerbera hybrida* homolog for the gibberellic acid stimulated transcript 1 [*GAST1*], from tomato) from a library representing late stages of corolla development in *Gerbera hybrida*. It was successfully shown that *GEG* expression in corollas and carpels coincides spatiotemporally with flower opening. In corollas and styles, *GEG* expression is temporally correlated with the cessation of longitudinal cell expansion. In plants constitutively expressing *GEG*, reduced corolla lengths and carpels with shortened and radially expanded stylar parts with concomitant reduction of cell expansion in these organs was observed and in styles, an increase in radial cell expansion was detected.

Due to the high degree of regularity of flower organ shape, the present inventors assumed that it would be useful to study the basis of organogenesis in plants using molecular and genetic approaches. In gerbera (*Gerbera hybrida*: Asteraceae), the most prominent part of the corolla is the blade like ligule, which has resulted from a fusion of three petal lobes (Bremer, K, Asteraceae, Cladistics & Classification, Portland, Timber Press, 1994; Helariutta, Y., et al., Plant Mol. Biol. 22, 183-193, 1993).

The styles of carpels are fine and elongated non-photosynthetic structures. By using differential hybridization of a corolla cDNA library a cDNA, *GEG* (for gerbera *GAST1*-like gene) was isolated and it was shown that *GEG* plays a role in the regulation of cell shape during corolla and carpel development in gerbera. *GEG* expression both spatially and temporally correlates with the opening of the corolla and with cessation of corolla elongation. In the carpel, induction of *GEG* expression coincides with the cessation of style elongation. In transgenic plants constitutively expressing *GEG*, corollas are shorter when compared to those of non-transformed lines. Similar to the corolla, constitutive *GEG* expression causes shortening of the carpel, but also a concomitant radial expansion of the style. In the present invention it is shown that epidermal cells of both the ligular part of the corolla and the style are reduced in length along organ axes. Radial expansion of the epidermal cells in styles was also observed. The results suggest that said phytohormone mediated cell expansion also can be applied in agriculture and forestry in order to provide new methods and means for controlling plant growth in a desired direction.

In a database search it was shown that *GEG* belongs to a gene family encoding putative small cell wall proteins with a cysteine-rich domain and a putative signal peptide sequence (GAST1 of tomato, GASA1-4 of *Arabidopsis* and GIP of petunia and RSI-1 of tomato) (Shi, L., et al., Plant J. 2, 153-159, 1992; Taylor, B. H., et al., Mol. Gen.

Genet. 243, 148-157, 1994; Herzog, M., et al. Plant Mol. Biol. 27, 743-752, 1995; Ben-Nissan, G., et al., Plant Mol. Biol.32, 1067-1074, 1996). *GEG* expression was experimentally induced by a treatment with gibberellic acid (GA_3), which is similar to previous reports indicating that these genes are susceptible of being regulated by gibberellic acid or auxin. The results obtained indicate that *GEG* is part of a phytohormone-mediated cell expansion mechanism that functions during corolla and carpel development and that mechanism can be used for developing new methods and means for providing plant raw materials with more desired and advantageous structure for agricultural and forestral applications.

The expression pattern of *GEG* correlates with completion of organ and cell elongation both in corollas and carpels. Furthermore, in transgenic plants, constitutive *GEG* expression demonstrates that excessive GEG production is able to cause alterations in organ and cell shape during corolla and carpel development. This suggests that *GEG* plays a role in determining cell shape during carpel and corolla morphogenesis, thus providing functional information for the role of *GEG*-like genes in plants.

In carpels, constitutive *GEG* expression reveals a negative interrelationship between longitudinal and radial growth. As described above, this is also evident in epidermal cells. However, in corollas, no radial expansion of epidermal cells, due to constitutive *GEG* expression, was observed. Furthermore, in carpels, no increase in style width was observed during endogenous *GEG* expression stage (Figure 4D). This would suggest that the primary role of GEG is to inhibit cell elongation. According to this hypothesis, constitutive *GEG* expression prematurely inhibits cell expansion in the longitudinal direction. This could lead to growth potential of the cell to be directed passively in the radial direction as seen in the epidermal cells of the style. The alternative hypothesis that GEG would promote radial and inhibit longitudinal expansion simultaneously is also possible.
In the present invention the dimensions of epidermal cells by scanning electron microscopy has been measured and *GEG* expression was observed in the underlying parenchymatic cells. This observation indicates that it is probable that analogous changes in cell shape can occur also in other plant cells The parallels between cell expansion and organ expansion are evident, although it is possible that in addition to cell expansion, cell division events could also contribute to the determination of the final shape at the stages analyzed.

The genes orthologous to *GEG* have been described earlier in various plants, e.g. *GAST1* (tomato), *GASA1-5* (*Arabidopsis*), *GIP* (petunia), and *RSI-1* (tomato) (Shi, L., et

al., Plant J. 2, 153-159, 1992; Taylor, B. H., et al., Mol. Gen. Genet. 243, 148-157, 1994; Herzog, M., et al. Plant Mol. Biol. 27, 743-752, 1995; Ben-Nissan, G., et al., Plant Mol. Biol.32, 1067-1074, 1996).

The *GEG*-like gene/protein family shares several features that may suggest a role for GEG in regulating cell expansion. Based on our studies of *GEG* expression and the fact that several members (*GEG*, *GAST1*, and *RSI-1*) have been isolated based on a differential screening method, we can conclude that the mRNA is relatively abundant, characteristic of a structural role for the gene product. Furthermore, the putative signal sequence and the absence of other targeting signals suggest that the gene products are secreted, possibly to the cell wall (Shi, L., et al., Plant J. 2, 153-159, 1992). Another characteristic feature is regulation of gene expression with phytohormones. The variability in the effective hormone indicates that the role of the genes may be downstream of various signal transduction pathways after their convergence.

Taken together with the data from transgenic plants suggesting that the primary role of GEG may be inhibiting axial cell expansion, it is possible that the GEG like function may be generally related to establishing cell wall properties during organogenesis in plants.

The present invention is further described in the following part in which the methodology and results are described in detail. These methods as well as the results obtained should not be interpreted as restricting the scope of the protection. Based on said description those skilled in the art can easily think of develop other equally well functioning desirable and advantageous applications for agriculture and forestry.

Example 1
Plant material

Gerbera hybrida var Terra Regina used in this research was obtained from Terra Nigra BV, Holland. The control and transgenic plants were grown under identical conditions (side by side) at the same time and the age of plants was same. Developmental stages of the inflorescence are described in Helariutta, Y., et al., Plant Mol. Biol. 22, 183-193, 1993. For all analyses, samples were collected from outermost ray florets (flowers) of the inflorescence, and each transgenic and control plant sample was harvested and treated at the same time.

Example 2
Plant transformation

Gerbera transformation was performed using *Agrobacterium tumefaciens*-mediated gene transfer as described previously (Elomaa, P., et al., Bio/technology 11, 508-511, 1993). Transformation was verified by RNA blot analysis showing *GEG* expression in leaves and by DNA blot analysis. The analyses have been performed on clones of the original transgenic plants (T_0).

Example 3
Isolation of plant DNA and RNA

Plant DNA was isolated using the methods by Dellaporta S. L., Plant Mol. Biol. Rep. 1, 19-21, 1983. Total RNA was isolated as described in Jones, J. D. G., et al., EMBO J. 4, 2411-2418, 1995 or by the Rneasy Plant total RNA kit (Qiagen, Chatsworth, CA). Poly(A)$^+$ RNA was isolated using oligo(dT) cellulose affinity chromatography (Sambrook, J., et al., Molecular Cloning: A Laboratory Manual., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989).

Example 4
Construction and differential screening of a corolla cDNA library

Polyadenylated RNA (5 μg), extracted from proximal part of ray floret corollas at developmental stages from 5 to 9 (Helariutta, Y., et al., Plant Mol. Biol. 22, 183-193, 1993) was used to construct a cDNA library in the ZAPII vector (ZAP-cDNA synthesis kit; Stratagene, La Jolla, CA). From the non amplified cDNA library, about 50.000 plaques were plated and transferred onto replica nylon membranes, and then screened differentially with radiolabeled first-strand cDNA pools from the ray floret tube region of the proximal part and distal part of ligule (first strand cDNA synthesis kit; Amersham).

GEG cDNA was isolated as a clone which is expressed stronger in proximal part than distal part of the ligule. Two independent, but similar cDNA clones were isolated, subcloned into pUC18 derivative, and sequenced using the AutoRead kit (Pharmacia, Uppsala, Sweden). The 813 bp genomic fragment containing part of *GEG* promoter was obtained by applying a 5' RACE like PCR amplification on genomic DNA. The *GEG* cDNA sequence and sequence of the 5' flanking region of the *GEG* gene have been submitted to EMBL database and the accession numbers are AJ005206 and AJ006273, re-

spectively.

Example 5
RNA blot analyses and in situ hybridization

Fifteen micrograms of total RNA was loaded per lane. The amount of RNA to be loaded was measured spectrophotometrically and the equal loading was confirmed by ethidium bromide staining of rRNA bands. The electrophoresis and hybridizations were made as described in Sambrook et al., 1989. The 259-bp long 3' fragment (of which 234 bp is from non coding region) served as the probe. Washing conditions of 0.2 SSC (1 SSC is 0.15 M NaCl and 0.015 M sodium citrate) and 0.1 % SDS, 58°C were applied in all RNA blots. In situ hybridization was carried out as described previously in Kotilainen M., et al., Plant Mol. Biol. 26, 971-978, 1994 using ^{35}S-CTP-labeled antisense and sense (control) RNA probes. The probes were transcribed from the same fragment as used in the gel blot studies under the T7 promoter in vector pSP73 (SP6/T7 transcription kit; Roche Diagnostics, Mannheim Germany).

Example 6
Scanning electron microscopic analysis

Corolla and carpel samples of control and transgenic plants were collected and further treated side by side at the same time. They were fixed in FAA buffer (50 % ethanol, 5 % acetic acid, and 2 % formaldehyde) overnight, and then transferred through ethanol series to 100 % ethanol, critical point dried (Balzers CPD 030 Critical Point Dryer, Bal-Tec, Liechtenstein) and coated with platinum/palladium (Agar Sputter Coater, Agar Scientific Ltd, UK). Specimens were mounted on aluminium stubs using graphite adhesive or tape, examined with scanning electron microscope (Zeiss Digital Scanning Microscope DSM 962, Karl Zeiss, Germany) in the Electron Microscopy Laboratory of the Institute of Biotechnology, University of Helsinki.

Example 7
Organ and cell measurements and statistical analysis

Organ length and width measurements were done with vernier caliper *in vivo,* except for carpel width, which was measured from scanning electron micrographs 200 μm below stigma. Cell length and width were measured by using scanning electron micrographs.

To study whether the differences in organ and cell expansion caused by constitutive *GEG* expression are statistically significant, Student s *t* tests and/or Rank sum tests were performed. Parametric *t* test was used if the normality and the equal variances of samples were confirmed (P values to reject < 0.050). Non parametric Rank sum test was used if either was not confirmed. The level of confidence is $P < 0.001$ in all statistically significant differences mentioned in this study.

Example 8
Isolation of *GEG* cDNA by differential screening

Both spatially restricted anthocyanin pigmentation patterns Putative signal sequence and the absence of other targeting signals suggest Another characteristic feature is regulation of gene expression with phytohormones. The variability in the effective hormone indicates that the role of the genes may be downstream of various signal transduction pathways after their convergence. (Helariutta, Y., et al., Plant Mol. Biol. 22, 183-193, 1993) and gene product accumulation patterns (Helariutta, Y., unpublished) in various regions of the gerbera ray floret corolla indicate region-specific control of gene expression along the longitudinal axis of the corolla. We performed several differential screening schemes along this axis during the late stages of corolla morphogenesis to isolate genes with differential expression within the corolla. In this context, we isolated a cDNA as a clone whose expression is stronger in the proximal part of the corolla than in the distal part. Based on high similarity to *GAST1*-like genes, we have named this cDNA *GEG*.

Sequence comparisons show that *GEG* belongs to a family of genes that are transcriptionally regulated by phytohormones in different plants. The predicted GEG protein has high sequence similarity with proteins encoded by genes whose expression is induced by gibberellic acid (*GAST1* of tomato, *GASA1-4* of *Arabidopsis,* and *GIP* of petunia) or by auxin (*RSI-1* of tomato) (Shi, L., et al., Plant J. 2, 153-159, 1992; Taylor, B. H., et al., Mol. Gen. Genet. 243, 148-157, 1994; Herzog, M., et al. Plant Mol. Biol. 27, 743-752, 1995; Ben-Nissan, G., et al., Plant Mol. Biol.32, 1067-1074, 1996). All of the derived polypeptides have a putative signal sequence at their N termini, with cleavage sites predicted according to von Heijne, G., Nuclear acid research 14, 4683-4690, 1986. Because other targeting signals have not been identified, it has been proposed that these gene products are targeted into the extracellular space or into the cell wall. Among 60 C-terminal amino acids, there are 22 identical residues of which 12 are cysteines.

During DNA gel blot analysis, the probe (a 259-bp long 3' fragment of the *GEG* cDNA, 90% noncoding) recognized one or two bands at the stringency used for RNA gel blotting. This most probably indicates that the expression analysis results presented below correspond to transcription of a single locus, and that the two bands found in some digests were due to restriction length polymorphism in the heterozygous cultivar. During low stringency DNA gel blot analysis, the full-length *GEG* cDNA probe recognized more bands, suggesting that there is a small gene family of *GEG*-like genes in the gerbera genome (data not shown).

Example 9
***GEG* mRNA is abundant in corollas and carpels**

The developmental expression pattern of *GEG* was studied by using RNA gel blot analyses. The expression of *GEG* is highest in floral organs, in addition a faint signal was detected in RNA from leaf blades. Strong *GEG* expression was observed in corolla tissue (both tube and ligule regions) and carpels, with more moderate signals in the scape (floral stem) and the receptacle (terminal enlargement of floral stem) (Figure 1). To understand the role of GEG in plant development, we focussed on *GEG* expression in corolla and carpel.

GEG expression was studied temporally at different stages of ray floret corolla development (Figures 2A to 2F, and Helariutta, Y., et al., Plant Mol. Biol. 22, 183-193, 1993. The expression correlates temporally with opening of both individual florets and the whole inflorescence, being induced at stage 7 (Figure 2G). Because of the large size of ray floret corollas in gerbera, we were able to isolate RNA from various parts of the corolla over time and study the developmental induction pattern of *GEG* expression by using RNA gel blot analysis. The pattern is intriguing. Just prior to the opening of the flower - and unfolding of the corolla - the onset of *GEG* expression occurs almost simultaneously from both ends of the corolla. The very first signal can be seen in the proximal part of the corolla, more precisely in the joint region of tube and ligule (Figure 2H: stage 7). During opening, the proximal expression proceeds in both directions: basipetally into the tube and acropetally into the ligule. Almost simultaneous to the onset of proximal expression, *GEG* expression also starts from the distal end of the corolla. This expression proceeds basipetally towards the middle of the ligule, where both proximal and distal expression domains meet just as the corolla has opened (Figure 2H: stage 8). *GEG* expression continues at a high level until senescence takes place (data not

shown). *In situ* hybridization analysis of *GEG* expression pattern revealed that the transcript can be detected both in the mesophyll and in the epidermis during opening of the corolla (Figure 3B).

In carpels, *GEG* expression was studied by gel blotting using RNA from samples taken before and after the opening of ray florets at developmental stages 6 and 8, respectively (Figures 2C to 2F and Helariutta, Y., et al., Plant Mol. Biol. 22, 183-193, 1993). As shown in Figure 2G, the onset of *GEG* expression coincides with the opening of the florets. In the style, *GEG* mRNA was detected in the outer epidermis and in parenchyma (cortex), but not in transmitting tissues (Figure 3A).

Example 10

Temporal correlation of *GEG* expression with cessation of organ and cell elongation in corollas and carpels

Biometric analyses (Figure 4) of corolla growth show that before its opening, the corolla expands both longitudinally and laterally. Soon after opening, growth ceases in both directions (Figures 4A and 4B). Temporally, *GEG* expression follows tightly the cessation of corolla growth, and is detected everywhere in corolla tissues just after opening (Figure 2D to 2F, and 2H).

The temporal *GEG* expression pattern along the apical-basal axis of corolla made it important to analyze whether *GEG* expression correlates with cessation of cell elongation. Cell length was measured in the distal and central regions of corolla (Figure 2H, regions 7 and 5, respectively) just after stage 7 (7+) and at stage 8. At these stages, *GEG* mRNA is present in the distal region, but reaches the central region just prior the stage 8 (Figure 2H).Cell length measurements revealed that cells in the distal region do not elongate, whereas in the central region, axial cell elongation takes place (Figure 5A). The cell length differences between stage 8 middle cells and other groups are statistically significant (Rank sum tests, $P < 0.001$). Cell width growth was detected both in distal and middle parts of corolla between stages 7+ and 8 (Figure 5 B).Thus, *GEG* expression strictly correlates with the cessation of cell expansion along the apical-basal axis.

Similar to corolla, carpel organogenesis was characterized in more detail using biometric analyses (Figure 4). Carpel length and width in the outermost ray florets were measured at various stages of inflorescence development. The opening of ray floret corollas and the whole inflorescence coincides with a change in the longitudinal expansion of carpels.

Elongation of the carpels takes place before the opening of the floret, being most rapid just prior to the opening. After opening, the elongation of carpels has ceased (Figures 2F and 4C). In the radial direction, the styles do not expand statistically during the elongation period or later (Figure 4D). Thus, in carpels the cessation of elongation is also temporally correlated with *GEG* expression.

Cell elongation coincides with the patterns described in organs, the epidermal cells of the style elongate before the opening of the floret but not later (Figure 4E). Therefore, cell elongation, at least to a large degree, is responsible for the observed carpel growth described above. In both corollas and carpels, *GEG* expression correlates temporally with cessation of cell expansion along the apical-basal axis.

Example 11
Transgenic plants that overexpress *GEG* have shorter corollas

Detailed temporal analysis of the developmental regulation of *GEG* shows that its transcription correlates with cessation of cell elongation both in the corolla and in the carpel. Based on this observation, we hypothesized that the functional role of the GEG polypeptide is to suspend cell elongation. To test this hypothesis, we generated transgenic plants in which *GEG* expression was under the control of a constitutively active promoter. In these plants, constitutive *GEG* expression should lead to premature inhibition of cell elongation, and to shorter organs with shorter cells.

The *GEG* cDNA was introduced into gerbera plants under regulation of the Cauliflower mosaic virus 35S promoter via *Agrobacterium tumefaciens*-mediated transformation. Four constitutively *GEG*-expressing lines were generated, and analyses of both the length and the width of 20 outermost ray floret corollas in four transgenic plants and control plants were conducted at developmental stage 9 when corolla growth has ceased. All four lines constitutively expressing *GEG* (m_1, m_3 , m_2, and m_5) have shorter corollas when compared to the non transformed line and the two control lines transformed with *GEG* in an antisense orientation, resulting in no or modest decline in *GEG* expression (Figures 6 and 7A; antisense lines with significantly reduced *GEG* expression levels were not obtained). The differences are statistically significant (*t* test/Rank sum test; $P < 0.001$). In contrast, the corolla width in all plants of lines constitutively expressing *GEG* lines remained unchanged when compared to control lines (Figure 7B).

Example 12
Constitutive expression of *GEG* decreases cell length in corollas

More detailed analyses of corolla and carpel phenotypes were performed with two transgenic lines m_1 and m_3 together with a non transformed control line as presented below. Constitutive expression of *GEG* in these transformants was verified by in situ hybridization of corolla cross-sections at developmental stage 6, when endogenous *GEG* expression is not yet present and by RNA gel blot analysis of leaf tissues where the endogenous expression is very low. In situ analyses show that all cell types overexpress *GEG* (data not shown).

In the corolla of both m_1 and m_3 lines together with the control line, the length and the width of epidermal cells were measured at the central part of the proximal end of the ray floret corolla ligules on their adaxial sides at developmental stage 8 (Figure 8A). In plants constitutively expressing *GEG*, cell length was reduced in a statistically significant manner (*t* test; $P < 0.001$) but no difference in cell width could be measured (Figures 8B, 8C, 9A, and 9B). As a conclusion, the major impact of constitutive *GEG* expression is the cessation of the axial cell expansion of epidermal cells in the ligule.

Example 13
Epidermal cells of the style are shorter and wider in lines constitutively expressing *GEG*

Compared to the control line, transgenic lines constitutively expressing *GEG* has a decrease in carpel length and an increase in carpel radius (Figures 10, 11A, and 11B). A comparison of cell length and the width of style epidermal cells between m_1 and m_3 lines constitutively expressing *GEG*, and the control line revealed a change in elongation pattern. Even before endogenous expression, at stage 6, statistically significant changes of cell length and width could be detected (*t* test; $P < 0.001$). In m_1 and m_3 lines constitutively expressing *GEG*, cell length was reduced and the width was increased compared to control line (Figures 10, 11C, and 11D). The constitutive expression phenotypes support the view that the *GEG* gene product regulates cell expansion in the axial dimension during carpel development as well as during corolla development. However, in the carpel, unlike in the corolla, we observed a concomitant opposite effect in the radial dimension.

Example 14
Gibberellic acid and regulation of *GEG* expression in the corolla

Because all of the homologous genes (see Introduction) are induced by phytohormones and because expression is spatially and temporally regulated, we studied whether *GEG* expression reacts to GA_3. Exogenous GA_3 application upregulated *GEG* expression in ray floret corollas of detached inflorescences (Figure 12). A short pulse of GA_3 was able to induce *GEG* in 2 hrs. However, because maximal levels of *GEG* mRNA were seen not before than 24 hrs after GA_3 application, it is possible that GA stimulation of *GEG* expression is indirect or decline of gibberellic acid concentration (rather than increase) induces *GEG* expression.

We have also isolated the genomic 5' flanking sequence of *GEG*. It contains two sequence motifs that are found in the flanking regions of rice and barley -amylase genes whose expression is regulated by gibberellic acid (Huang, N., et al., Plant Mol. Biol. 14, 655-668, 1990; Skriver, K., et al., Proc. Natl. Acad. Sci. USA 88, 7266-7270, 1991. This further supports the idea that *GEG* expression is developmentally regulated by gibberellic acid.

Figure 1. RNA gel blot hybridisation analysis showing the tissue specificity of *GEG* expression.

Autoradiography of an RNA gel blot probed with a 259-bp long 3' fragment of the *GEG* cDNA (90 % noncoding). Fifteen micrograms of total RNA was loaded per lane and equal loading was confirmed by ethidium bromide staining. Organs covering several developmental stages were examined. Scape, floral stem; receptacle, terminal enlargement of floral stem.

Figure 2. Analysis of *GEG* expression during corolla and carpel development.

(A) to (F) Different developmental stages of gerbera inflorescence, according to Helariutta et al. (1993).

(A) The developmental stage is 1, (B) 3, (C) 5, (D) 7, (E) 7.5, and (F) 8.
(G) The expression of *GEG* in carpel and corolla correlates with opening of both individual ray florets and the whole inflorescence.

Spatial partition of ray floret corolla. Regions are indicated above the gel. The onset of expression occurs from both ends of the corolla (stage 7, regions 2 and 7) and just as the corolla has opened. Both expression domains meet at the middle of the ligule (stage 8, region 5). Region 1 is the tubular part of ray floret corolla (tube); region from 3 (proximal region) to 7 (distal region) represent the ligular part of the corolla. *GDFR1* is used as a loading control. The developmental stages are the same as presented in (A) to (F).

Figure 3. Analysis of *GEG* expression in carpel and corolla by in situ hybridization.
In both organs (stage 7.5), *GEG* expression is seen in epidermal and parenchymatic cells as white silver grains. *In situ* analysis were carried out using the ^{35}S-CTP-labeled antisense and sense (control, data not shown) RNA probes. The probes were transcribed from the same 3' fragment of *GEG* cDNA as used in RNA gel blot analysis.
(A) Cross-section of carpel style.
(B) Cross-section of marginal region of proximal part of corolla ligule.
Bar = 100 μm for (A) and (B).

Figure 4. Biometric analysis of corolla and carpel length and width development.
(A) and (B) Corolla length and width, respectively: Temporal *GEG* expression tightly follows the cessation of corolla expansion in both longitudinal and lateral directions (see also Figure 2) .
(C) Carpel length: In the carpel, cessation of elongation temporally correlates with *GEG* expression.
(D) Carpel width: The carpel styles do not expand in radial direction during the elongation period or later.
(E) Carpel cell length: In carpel the epidermal cells of style elongate before opening of the floret but not later, thus the retardation of cell elongation correlates with *GEG* expression.
Timing of different developmental stages (described in Helariutta, Y., et al., Plant Mol. Biol. 22, 183-193, 1993) was measured by following the development more than 50 inflorescences under our standard greenhouse conditions. The lengths and widths of both corolla and carpel, shown in (A) to (D), were measured from 15 to 30 outermost ray florets at each developmental stage. Samples were collected at least from two different inflorescences. Carpel cell length (E) of 72 epidermal cells of each time point was measured. Cell lengths of 18 epidermal cells 200 - 400 μm below the stigma of each carpel were determined, and the average cell length of four carpels was measured. Numbers below the curves correspond to the developmental stages of inflorescence (see Figures 2A to 2F). Error bars indicate the standard deviation.

Figure 5. Cell length and width in distal and central regions of ray floret corolla just prior and after the opening (stages 7+ and 8, respectively).
GEG is expressed in proximal part at both stages, whereas *GEG* expression reaches the central region just prior the stage 8 (see Figure 2H).
(A) Corolla cell length in microns. The cells in distal region do not elongate, whereas the cells in central region elongate. Thus, *GEG* expression strictly correlates with cessation of cell expansion along the apical-basal axis. Tip, distal region; Mid, central region.
(B) Cell width (μm) growth was detected both in distal and central regions of corolla.
Cell length and width were measured with vernier caliper using scanning electron micrographs. The length of forty cells in three distinct ray floret corollas each was measured at each point. At distal region of corolla the measurements were done at 1 mm from the tip. Cell widths were measured by counting cell numbers on 36 of 570 μm long transverse lines and counting the average cell widths of each line. Error bars indicate the standard deviation.

Figure 6. Comparison of the ray floret corollas of a non transformed line (wt) with that of an m_3 plant constitutively expressing *GEG* before (stage 7), during (7.5), and after (9) the opening of ray florets.

Figure 7. Analysis of corolla length and width for four lines constitutively expressing *GEG* and the control lines.
(A) Corolla length (mm). Length of the outermost ray floret corollas of wild-type, constitutively *GEG* expressing lines (m_1, m_3, m_2, and m_5), and two *GEG* antisense lines with no (t_4) or a modest (t_9, 80% remaining) decline of *GEG* expression. All four lines constitutively expressing *GEG* have statistically shorter corollas compared to the control lines.
(B) The corolla width (mm) of all lines constitutively expressing *GEG* remained unchanged when compared to control lines.
Forty outermost ray floret corollas of two inflorescences of each line at stage 9 were collected. Corolla width and length of four lines constitutively expressing *GEG* (m_1, m_3, m_2, and m_5), a non transformed line and two antisense lines (with no or modest effect in *GEG* expression) were measured. Error bars indicate the standard deviations.

Figure 8. Analysis of the effects of constitutive *GEG* expression on corolla epidermal cells.

(A) The epidermal cells are organized into longitudinal files running along the apical-basal axis of corolla.
(B) Scanning electron microscopy of the adaxial (upper) side of proximal part of ray floret corolla of a non transformed control line (stage 8). The area in (B) is marked with a white box in (A). One of the epidermal cells is highlighted.
(C) Scanning electron microscopy of the corresponding region of an m_1 line constitutively expressing *GEG*. Bar in (C) = 75 μm for (B) and (C).

Figure 9. Cell lengths and widths in the same regions as described in Figure 8 of m_1 and m_3 lines constitutively expressing *GEG* and a control line (wt). In plants constitutively expressing *GEG*, cell length was reduced but no difference in cell width could be measured.
(A) Corolla cell length in microns. The cell lengths in these two lines constitutively expressing *GEG* are shorter when compared to control and the differences are statistically significant.
(B) Corolla cell width in microns. Cell widths of these lines constitutive expressing *GEG* did not differ from that of a control line.
Three ray floret corollas of transgenic lines m_1 and m_3 together with a non transformed control line were collected (stage 8), and cell length and width were measured at the region marked with a white box in the Figure 8A. In corollas, transverse lines were drawn on micrographs, and cells were chosen at intervals of 1 cm for length measurements. Cell length of about 200 cells were measured of m_1, m_3, and a control line. Cell width was measured by counting cell numbers on 30 - 36 of 570 μm transverse lines and counting the average cell width of each line (approximately 40 cells per line).

Figure 10. Scanning electron microscopy of the epidermis of stylar part of the carpel 300 m below the stigma. In an m_1 line constitutively expressing *GEG*, cell length was reduced and the width was increased compared to control line.
(A) A non transformed control line and, (B) an m_1 line constitutively expressing *GEG* at stage 6. Bar = 100 μm for (A) and (B).

Figure 11. Analysis of the effects of constitutive *GEG* expression on epidermal cells of the carpel style.
(A) Carpel length in millimeters. Carpel length of lines constitutively expressing *GEG* is shorter when compared to a control line.
(B) Carpel width in microns. Compared to a control line, carpel radius has increased in lines constitutively expressing *GEG*.

(C) Carpel cell length in microns. Comparison of cell length of m_1 and m_3 to a non transformed control line reveals that epidermal cells of style of lines constitutive expressing *GEG* are shorter when compared to a control line.
(D) Carpel cell width in microns. The epidermal cells of constitutively *GEG* expressing lines are wider when compared to a control line.
Twelve to twenty carpels of two lines constitutively expressing *GEG* (m_1 and m_3) and a non transformed control line were measured. Carpel length was measured at developmental stage 9; carpel width, epidermal cell length, and width were measured at stage 6. Cell length of 18 individual epidermal cells 200 - 400 μm below the stigma of each carpel and the average cell length of 12 - 20 carpels of each line were measured. The differences presented in (A), (B), (C) and (E) are statistically significant. Error bars indicate the standard deviations.

Figure 12. Exogenous gibberellic acid application upregulates *GEG* expression in ray floret corollas
RNA gel blot showing *GEG* expression after the addition of GA_3 just before the opening of the inflorescence (stage 7+). The scape (floral stem) was cut 5 cm below inflorescence.
(A) The control inflorescences were grown on 50 mM sucrose.
(B) Induction of *GEG* expression was detected when inflorescences were grown on 50 mM sucrose with 5 μM GA_3.
(C) Induction of *GEG* expression was also detected when inflorescences were first incubated in 50 mM sucrose with 50 μM GA_3 for 5 min, and then transferred to 50 mM sucrose medium.
Approximately 10 ray floret corollas were collected for RNA isolation at each time point (hours) after GA_3 addition.

We claim:

1. Isolated and essentially purified nucleic acid sequences for altering the size and shape of plant cells or plant organs, c h a r a c t e r i z e d in, that the GEG-like nucleic acid sequences are capable of encoding GEG-like gene products which are substantially homologous to the gene product of the Gerbera GAST1-like cDNA, preferably its C-terminal domain comprising one or more cysteine residues, and said GEG-like nucleic acid sequences having the capacity of spatiotemporal control of the plant cell growth or of directing said capacity.

2. The nucleic acid sequences according to claim 1, c h a r a c t e r i z e d in, that the GEG-like nucleic acid sequences encode GEG-like gene products having a C-terminal domain (SEQ ID NO:1:), which is substantially homologous to the C-terminal part of gene product of GEG (SEQ ID NO:2:).

3. The nucleic acid sequences according to claims 1-2, c h a r a c t e r i z e d in, that the GEG-like nucleic acid sequences capable of hybridizing with SEQ ID NO:3: under defined conditions.

4. The nucleic acid sequence according to claims 1-3, c h a r a c t e r i z e d in that encodes a GEG-like nucleic acid sequences encode GEG-like gene products with a C-terminal domain comprising at least 4, preferably at least 8, most preferably at least 12 cystein residues.

5. The nucleic acid sequence according to claim 4, c h a r a c t e r i z e d in that the encoded C-terminal domain comprises 12 cystein residues.

6. The nucleic acid sequence according to claims 1-5, c h a r a c t e r i z e d in that it comprises nucleic acid sequences obtainable by modification of the nucleic acid sequences obtainable by conventional methods from *Gerbera hybrida,* GAST1 obtainable from tomato, GASA1-4 obtainable from *Arabidopsis,* GIP obtainable from petunia and RSI-1 obtainable from tomato using SEQ ID NO:3: or parts thereof.

7. The nucleic acid sequence according to claims 1-6, c h a r a c t e r i z e d in that it comprises SEQ ID NO:3: (GEG cDNA) obtainable from *Gerbera hybrida.*

8. A nucleic acid sequence, characterized in that it comprises the GEG-promoter sequence (SEQ ID NO:4:).

9. The GEG-like gene products expressed by the nucleic acid sequence according to claims 1-8 for altering the size and shape of plant cells or plant organs, characterized in that the GEG-like gene product is a polypeptide having C-terminal domain substantially homologous with SEQ ID NO:1:, comprising one or more able cysteine residues said GEG-like gene product having the capacity of spatiotemporal control of cell growth.

10. The GEG-like gene products according to claim 9, characterized in that the C-terminal domain comprises at least 4, preferably at least 8, most preferably at least 12 cystein residues.

11. The GEG-like gene products according to claims 10, characterized in that it encodes a GEG-like gene product with a C-terminal domain comprising 12 cystein residues.

12. The GEG-like gene products according to claim 9-11, characterized in that it comprises a polypeptide having an amino acid sequence substantially homologous with SEQ ID NO:1:, excluding the amino acid sequence SEQ ID NO:2:.

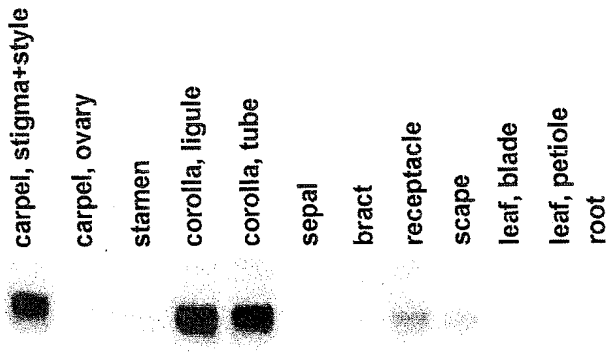

carpel, stigma+style
carpel, ovary
stamen
corolla, ligule
corolla, tube
sepal
bract
receptacle
scape
leaf, blade
leaf, petiole
root

Fig 1.

Fig.2A Fig.2B Fig.2C Fig.2D

Fig.2E

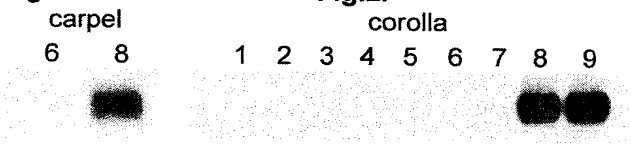
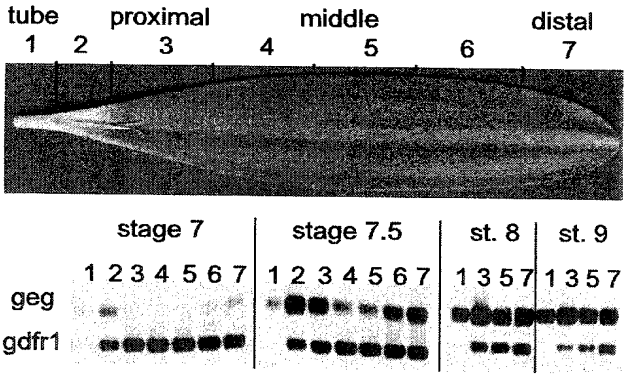


Fig.2F

Fig.2G

Fig.2H



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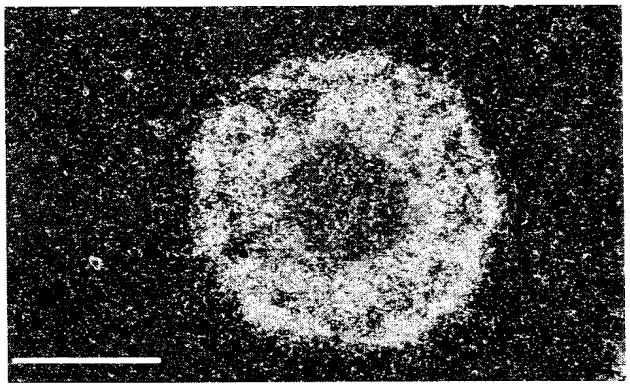

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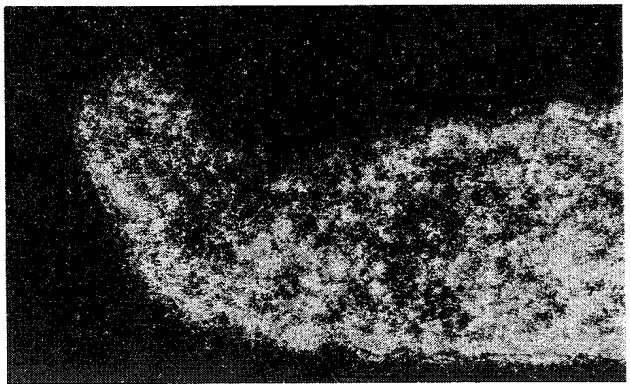

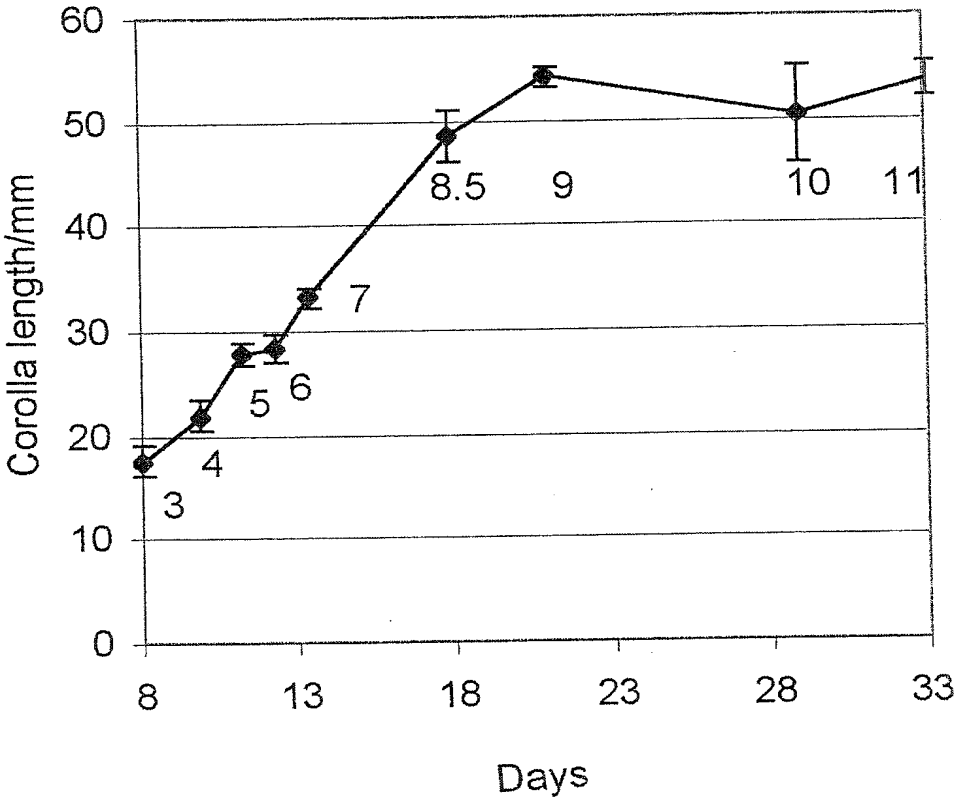
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40
30
20
10
0
Corolla length/mm
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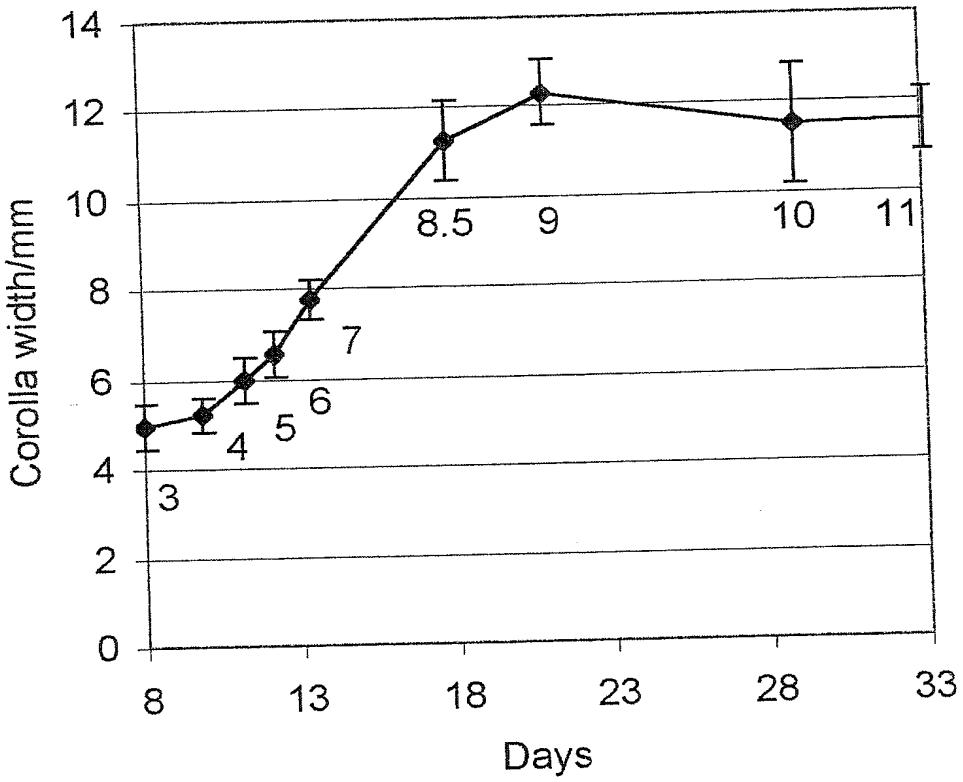
4
5
6
7
8.5
9
10
11
8
13
18
23
28
33
Days

Fig 4A.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Corolla width/mm
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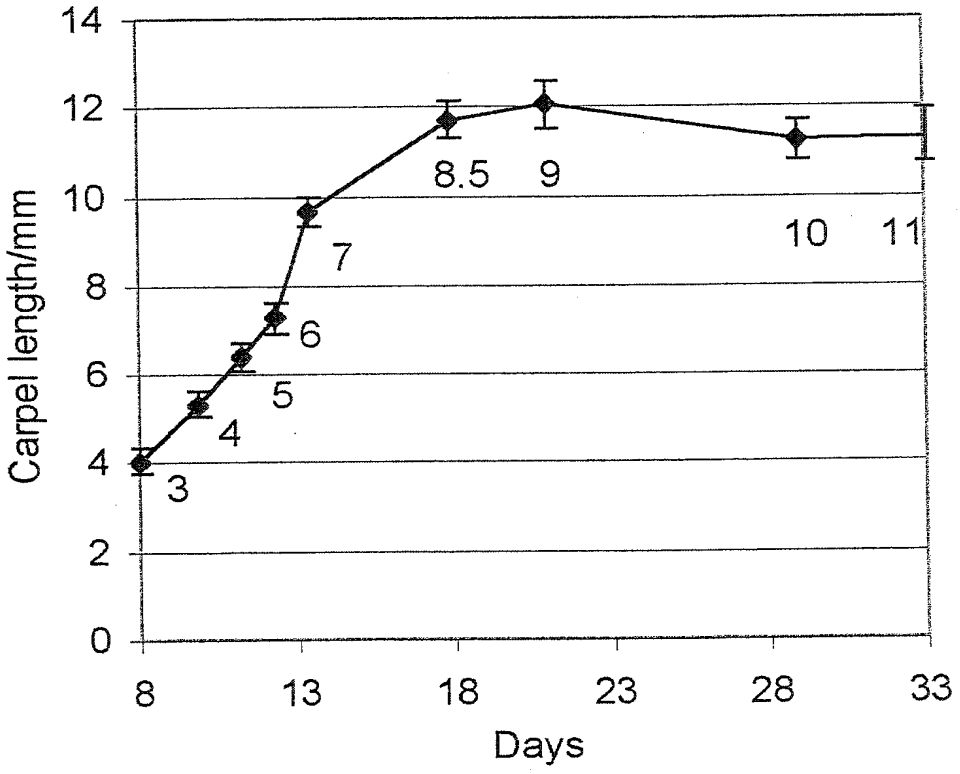
18
23
28
33
Days

Fig 4B.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Carpel length/mm
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Days
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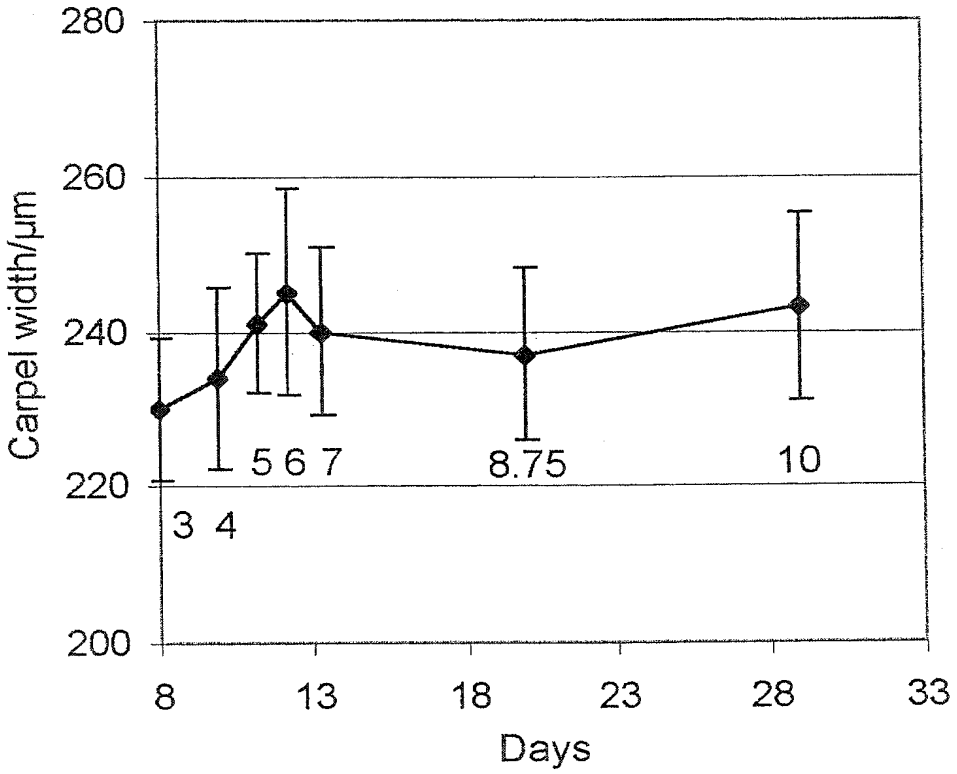
10
11

Fig 4C.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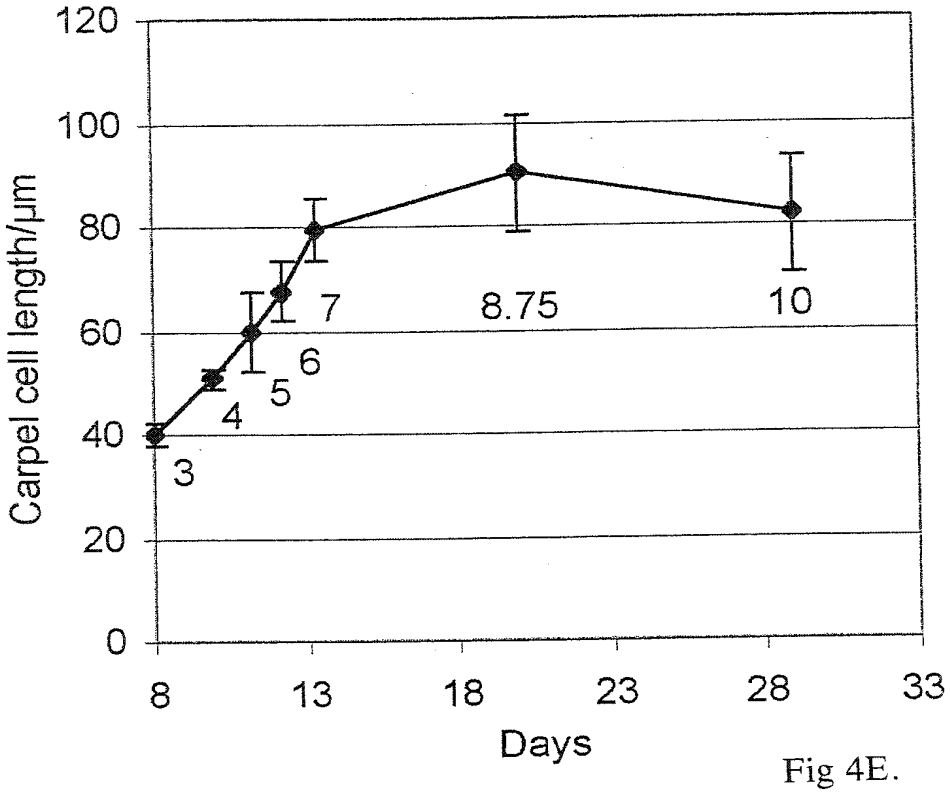
28
33
Days

Fig 4D.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20
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Carpel cell length/µm
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18
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28
33
Days
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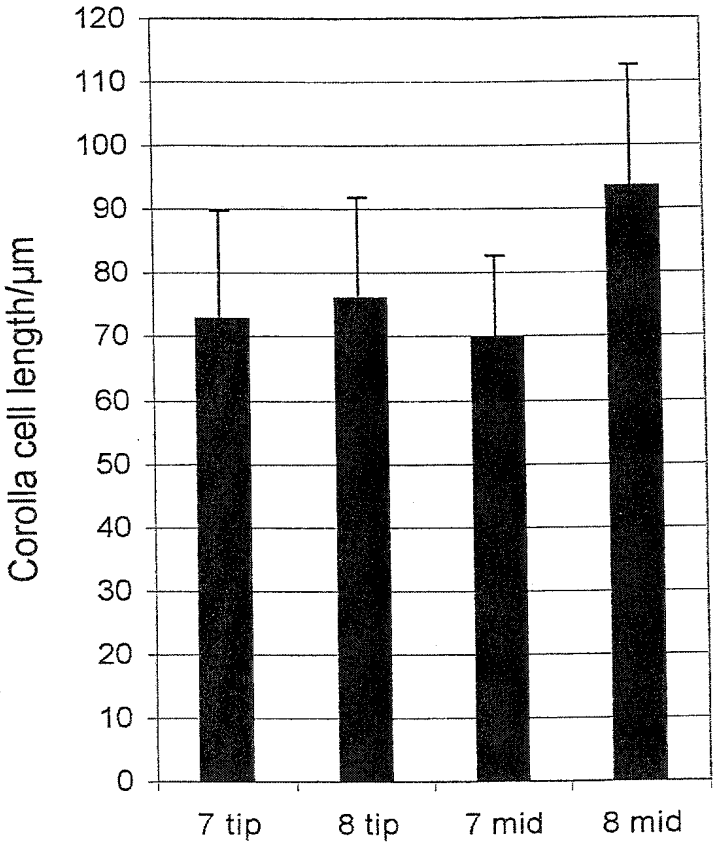
5
6
7
8.75
10

Fig 4E.


120
110
100
90
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40
30
20
10
0
Corolla cell length/µm
7 tip
8 tip
7 mid
8 mid

Fig 5A.

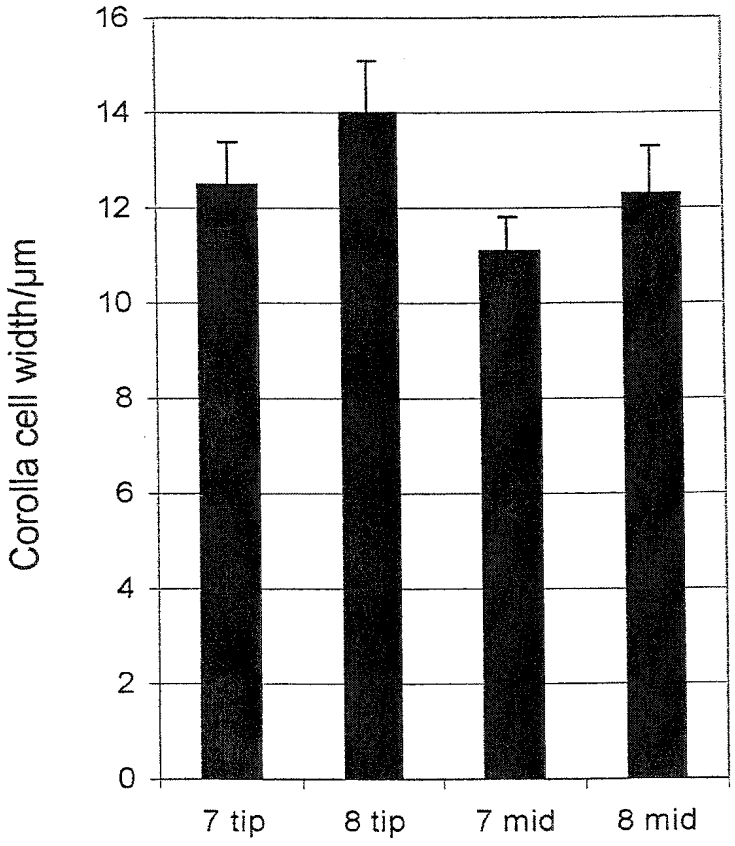
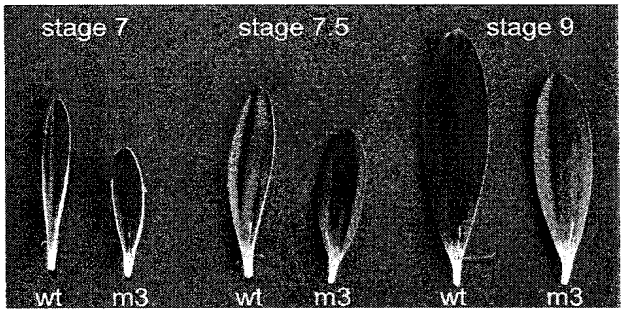
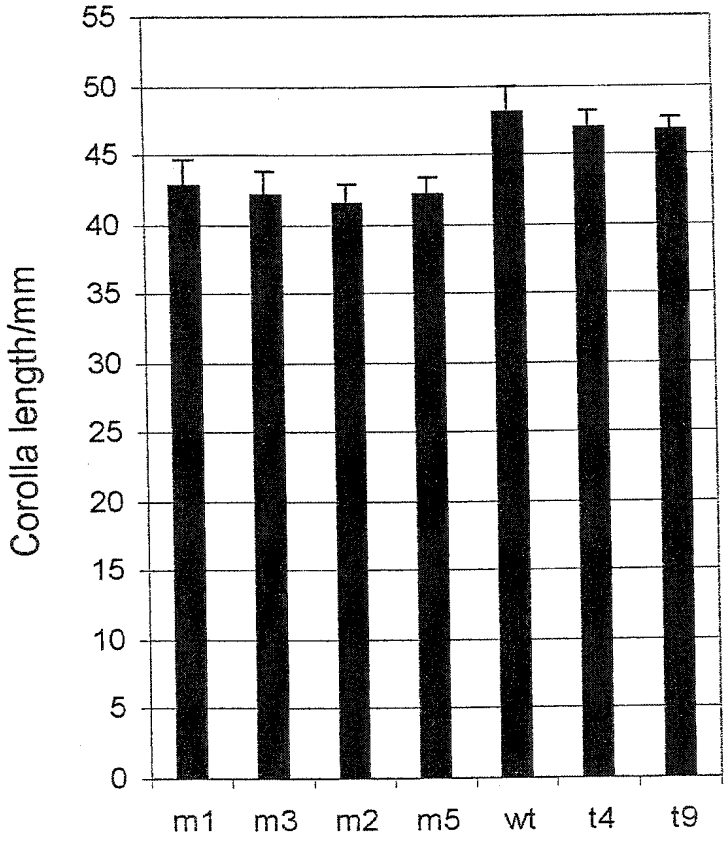


Fig 5B.

Fig.6




Corolla length/mm
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50
45
40
35
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25
20
15
10
5
0
m1
m3
m2
m5
wt
t4
t9

Fig 7A.

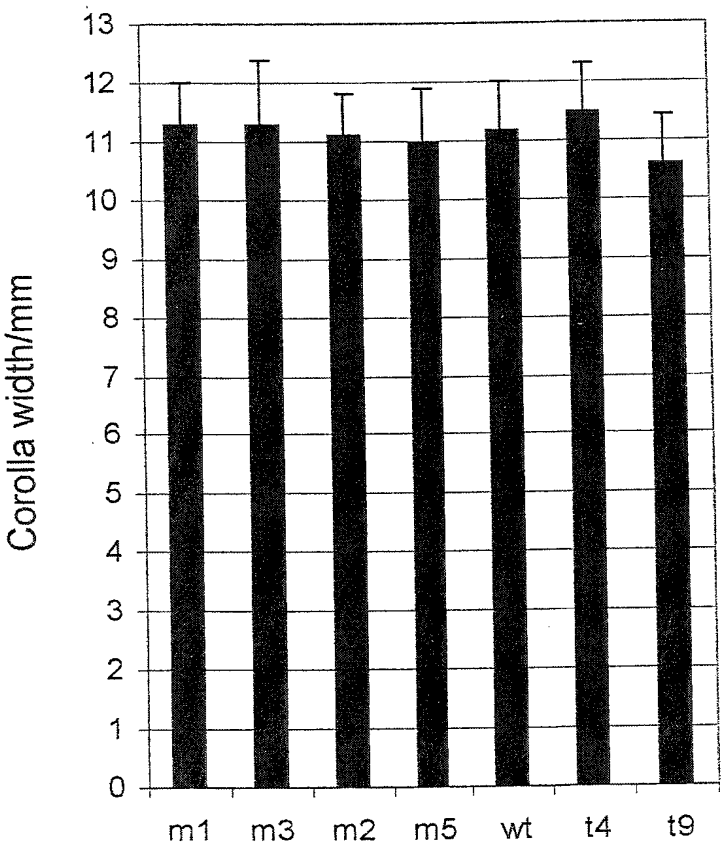


Fig 7B.

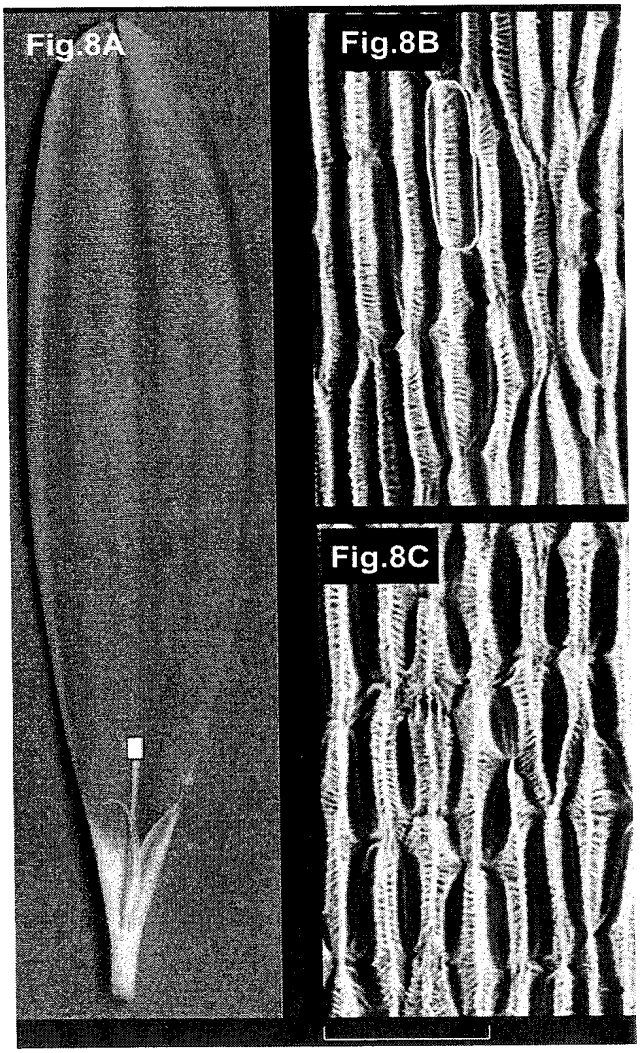


Fig.8A
Fig.8B
Fig.8C

Fig.9A

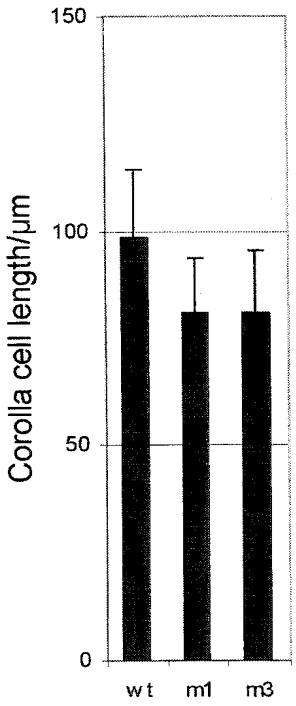

150
100
50
0
Corolla cell length/µm
wt
m1
m3

Fig.9B

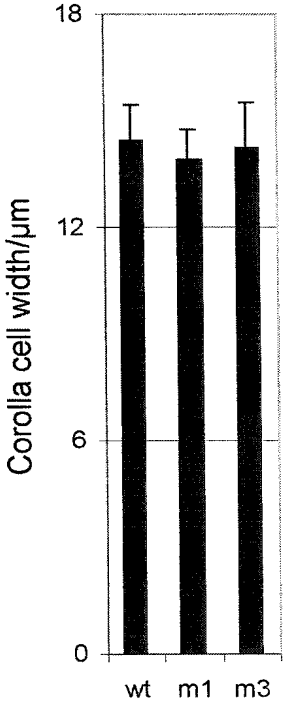

18
12
6
0
Corolla cell width/µm
wt
m1
m3

Fig.10A

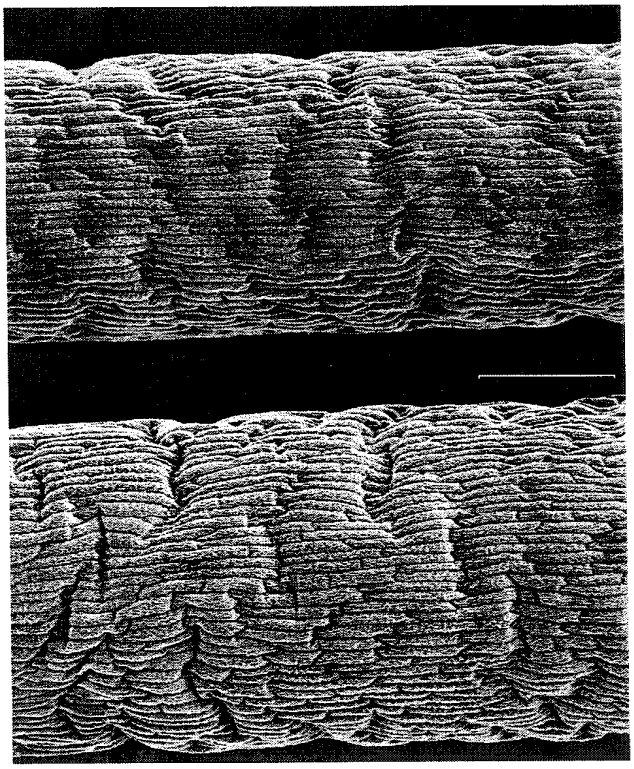


Fig.10B

Fig.11A

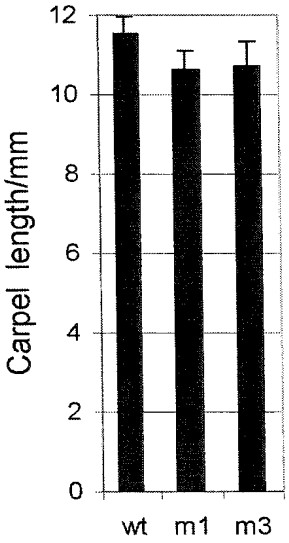


Fig.11B

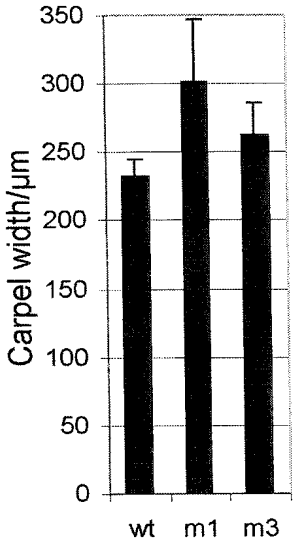


Fig.11C

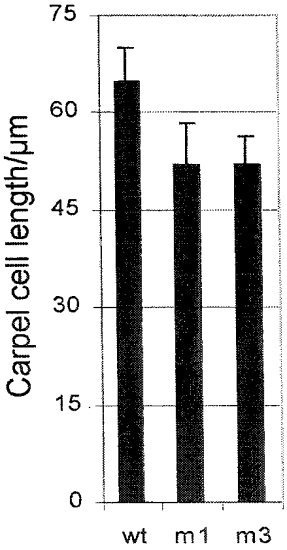
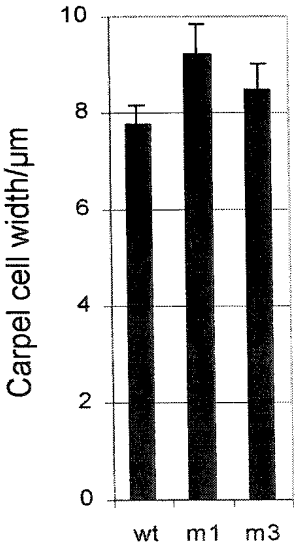


Fig.11D



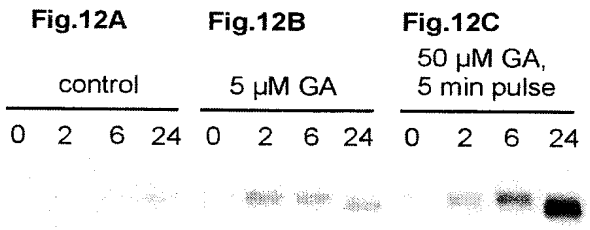
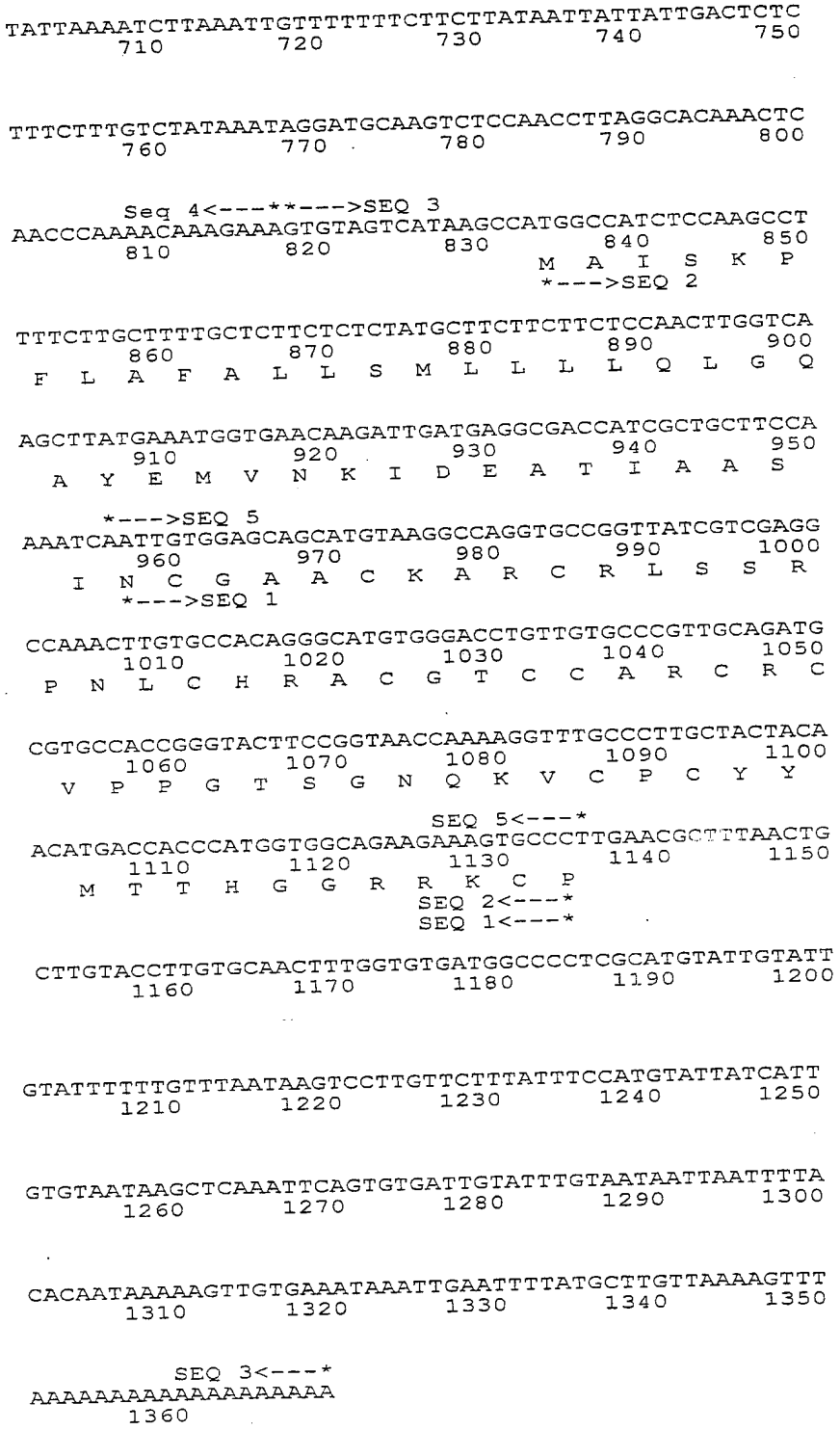


Fig.12A
Fig.12B
Fig.12C
control
5 µM GA
50 µM GA,
5 min pulse
0 2 6 24 0 2 6 24 0 2 6 24

Fig 13.

```
*--->SEQ 4
AATCAATCTTTGTAGGGAGCAATTTAGGATAGTTTAGTTCAGTATATGTC
         10         20         30         40         50

AATATATCTGATCGTTTGAGAAAAAAAGGATAATAAAGACGATAATATTC
         60         70         80         90        100

TTTATTTTCCGACCTAAAGAGTTAGGTAAGTTATAATTTACTTTAATCGC
        110        120        130        140        150

TCGGTGTTTAATGAAATATTATATCCAAGTCTCGACTCTCAATAAGTGTT
        160        170        180        190        200

AACATCTTTTTAGGTCGTATTTGCTTACCAAAAAACCTAAAATTTGTGTT
        210        220        230        240        250

CATATGCAGCGAACGCTTTACTGAATGATATAGATTAGTTGAGGCAAAGG
        260        270        280        290        300

TTACAATATCATGGTTATCAAAAAATAATATCTTCTCTTGTTATTCTTTC
        310        320        330        340        350

GTGAAAACTAAAGTCACCCAAGAACACGTTTTATACATGAGCCACTACGT
        360        370        380        390        400

TTGATAGTATAGTTTACCAACCGTTCCACTGTTACATACTGGATAGTACA
        410        420        430        440        450

TTTTATCAACCATTCCGCTGTCACAAAAAAAAAAAAAAAAATTAATACCG
        460        470        480        490        500

TCTTTTTAAAAGTCGATAAAAAGGATAACAAGGATAACAACCGTTTAATC
        510        520        530        540        550

ATCAAATTATTTGCAGTTAAGAGGTCATGAGAGATATCATCCTAGCTAAA
        560        570        580        590        600

TGCTTTTAGGTGAAACTCTTGTGGTGTCAATAACATTTAAATTTATAAAG
        610        620        630        640        650

AAAAAGAGAAATTATTAAAATCTTAAATTGTTTTTTTCAAAAAGAGAAAT
        660        670        680        690        700
```

Fig 13. (continued)



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: TEERI, Teemu, et al.
(B) STREET: Biotekniikan instituutti, Viikinkaari 9
(C) CITY: Helsinki, Helsingin yliopisto
(D) STATE: Viikin biokeskus
(E) COUNTRY: FINLAND
(F) POSTAL CODE (ZIP): FIN-00014
(G) TELEPHONE: 358-9-70859423
(H) TELEFAX: 358-9-70859570

(ii) TITLE OF INVENTION: METHODS AND GENE PRODUCTS FOR ALTERING THE SHAPE OF CELLS AND ORGANS IN PLANTS

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Gerbera hybrida

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Asn Cys Gly Ala Ala Cys Lys Ala Arg Cys Arg Leu Ser Ser Arg Pro
1               5                  10                  15

Asn Leu Cys His Arg Ala Cys Gly Thr Cys Cys Ala Arg Cys Arg Cys
            20                  25                  30

Val Pro Pro Gly Thr Ser Gly Asn Gln Lys Val Cys Pro Cys Tyr Tyr
        35                  40                  45

Asn Met Thr Thr His Gly Gly Arg Arg Lys Cys Pro
    50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ala Ile Ser Lys Pro Phe Leu Ala Phe Ala Leu Leu Ser Met Leu
1 5 10 15

Leu Leu Leu Gln Leu Gly Gln Ala Tyr Glu Met Val Asn Lys Ile Asp
20 25 30

Glu Ala Thr Ile Ala Ala Ser Lys Ile Asn Cys Gly Ala Ala Cys Lys
35 40 45

Ala Arg Cys Arg Leu Ser Ser Arg Pro Asn Leu Cys His Arg Ala Cys
50 55 60

Gly Thr Cys Cys Ala Arg Cys Arg Cys Val Pro Pro Gly Thr Ser Gly
65 70 75 80

Asn Gln Lys Val Cys Pro Cys Tyr Tyr Asn Met Thr Thr His Gly Gly
85 90 95

Arg Arg Lys Cys Pro
100

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 552 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTGTAGTCAT AAGCCATGGC CATCTCCAAG CCTTTTCTTG CTTTTGCTCT TCTCTCTATG 60

CTTCTTCTTC TCCAACTTGG TCAAGCTTAT GAAATGGTGA ACAAGATTGA TGAGGCGACC 120

ATCGCTGCTT CCAAAATCAA TTGTGGAGCA GCATGTAAGG CCAGGTGCCG GTTATCGTCG 180

AGGCCAAACT TGTGCCACAG GGCATGTGGG ACCTGTTGTG CCCGTTGCAG ATGCGTGCCA 240

CCGGGTACTT CCGGTAACCA AAAGGTTTGC CCTTGCTACT ACAACATGAC CACCCATGGT 300
GGCAGAAGAA AGTGCCCTTG AACGCTTTAA CTGCTTGTAC CTTGTGCAAC TTTGGTGTGA 360
TGGCCCCTCG CATGTATTGT ATTGTATTTT TTGTTTAATA AGTCCTTGTT CTTTATTTCC 420
ATGTATTATC ATTGTGTAAT AAGCTCAAAT TCAGTGTGAT TGTATTTGTA ATAATTAATT 480
TTACACAATA AAAAGTTGTG AAATAAATTG AATTTTATGC TTGTTAAAAG TTTAAAAAAA 540
AAAAAAAAAA AA 552

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "promoter"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

AATCAATCTT TGTAGGGAGC AATTTAGGAT AGTTTAGTTC AGTATATGTC AATATATCTG 60
ATCGTTTGAG AAAAAAAGGA TAATAAAGAC GATAATATTC TTTATTTTCC GACCTAAAGA 120
GTTAGGTAAG TTATAATTTA CTTTAATCGC TCGGTGTTTA ATGAAATATT ATATCCAAGT 180
CTCGACTCTC AATAAGTGTT AACATCTTTT TAGGTCGTAT TTGCTTACCA AAAAACCTAA 240
AATTTGTGTT CATATGCAGC GAACGCTTTA CTGAATGATA TAGATTAGTT GAGGCAAAGG 300
TTACAATATC ATGGTTATCA AAAAATAATA TCTTCTCTTG TTATTCTTTC GTGAAAACTA 360
AAGTCACCCA AGAACACGTT TTATACATGA GCCACTACGT TTGATAGTAT AGTTTACCAA 420
CCGTTCCACT GTTACATACT GGATAGTACA TTTTATCAAC CATTCCGCTG TCACAAAAAA 480
AAAAAAAAAA ATTAATACCG TCTTTTTAAA AGTCGATAAA AAGGATAACA AGGATAACAA 540
CCGTTTAATC ATCAAATTAT TTGCAGTTAA GAGGTCATGA GAGATATCAT CCTAGCTAAA 600
TGCTTTTAGG TGAAACTCTT GTGGTGTCAA TAACATTTAA ATTTATAAAG AAAAAGAGAA 660
ATTATTAAAA TCTTAAATTG TTTTTTTCAA AAAGAGAAAT TATTAAAATC TTAAATTGTT 720
TTTTTCTTCT TATAATTATT ATTGACTCTC TTTCTTTGTC TATAAATAGG ATGCAAGTCT 780
CCAACCTTAG GCACAAACTC AACCCAAAAC AAAGAAAGTG TAGTCATAAG CC 832

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 180 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(A) DESCRIPTION: /desc = "3' end of the cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AATTGTGGAG CAGCATGTAA GGCCAGGTGC CGGTTATCGT CGAGGCCAAA CTTGTGCCAC 60

AGGGCATGTG GGACCTGTTG TGCCCGTTGC AGATGCGTGC CACCGGGTAC TTCCGGTAAC 120

CAAAAGGTTT GCCCTTGCTA CTACAACATG ACCACCCATG GTGGCAGAAG AAAGTGCCCT 180

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 00/00475

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/29 // A01H 3/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	The Plant Cell, Volume 11, June 1999, Mika Kotilainen et al, "GEG Participates in the Regulaiton of Cell and Organ Shape during Corolla and Carpel Development in Gerbera hybrida" page 1093 - page 1104 --	1-12
X	National Library of Medicin (NLM), file Medline, Medline accession no. 97156145, Ben-Nissan G et al: "The petunia homologue of tomato gast1: transcript accumulation coincides with gibberellin-induced corolla cell elongation"; & Plant Mol Biol 1996 Dec; 32 (6): 1067-74 --	1-12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 Sept 2000	09 -10- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Hampus Rystedt/ELY Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 00/00475

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	National Library of Medicin (NLM), file Medline, Medline accession no. 99084756, Shi L et al: "Gibberellin and abscisic acid regulate GAST1 expression at the level of transcription"; & Plant Mol Biol 1998 Dec; 38 (6): 1053-50 --	1-12
A	National Library of Medicin (NLM) file Medline, Medline accession no. 93251105, Shi L et al: "Characterization of a shoot-specific, GA3-and ABA-regulated gene from tomato"; & Plant J 1992 Mar; 2 (2): 153-9 -- --------	1-12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI00/00475

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 3
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See extra sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI00/00475

It is not considered possible to search claim 3, for two reasons:

1. Claim 3 contradicts itself with regard to the preceding claims, as it claims a nucleic acid (NA) sequence that encode a protein that is encoded by NA sequence that hybridize to the claimed NA sequence, i.e. the NA sequence should hybridize to itself.
2. The "defined conditions" mentioned in the claim are not defined.

A search has been carried out for SEQ ID NO:3, but not for its complementary strand.